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14. ABSTRACT

Regulatory approvals, training of research assistants, generation of forms, and creation of the database were completed in Q1 and Q2. Thereafter, the major goals for Year 1 were to complete approximately one-half of Experiment 1 (EXP1) and all of the Experiment 2 Pilot Study (EXP2-pilot). Progress on EXP1 was better than projected, with research visits having been completed for 25 of 28 participants; batched analyses of serum and sweat samples were initiated. Progress was delayed because of 1) limited access to the temperature-controlled facility needed for this EXP1, and 2) hospital shortages of the product required for EXP2-pilot (i.e., calcium chloride or calcium gluconate). The benchmark for moving forward to EXP2 (i.e., 3 successful clamps) is expected to be achieved in October. Therefore, the application for approval of EXP2 will be submitted in Year 2 Q1. One unanticipated problem in EXP2-pilot was reported, but the local IRB subsequently determined that the event was not related to the experimental procedure. Because analyses of samples and data have not yet been completed for EXP1, no abstracts or manuscripts have been generated. The first abstract submission is expected in November 2013.

15. SUBJECT TERMS
calcium homeostasis, exercise, bone resorption, parathyroid hormone

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INTRODUCTION

The **Global Aim** of the proposed research is to investigate a novel mechanism for exercise-related bone loss. We postulate that the disruption of calcium (Ca) homeostasis during acute exercise is a trigger for the activation of bone resorption. The working model portends that excessive dermal Ca loss (i.e., sweating) causes a decline in serum ionized Ca (iCa; the unbound fraction) and triggers an acute increase in parathyroid hormone (PTH). PTH can defend serum Ca by reducing urinary Ca excretion, increasing intestinal Ca absorption, and increasing mobilization of skeletal Ca (bone resorption). If an increase in bone resorption occurs repeatedly over multiple exercise sessions (i.e., exercise training) and is not accompanied by appropriate loading forces to stimulate bone formation, we postulate that this could lead to a decrease in BMD over time. The **Specific Aims** are to **1)** determine whether the magnitude of dermal Ca loss during exercise is a determinant of the decline in iCa and increases in PTH and carboxy-terminal collagen crosslinks (CTX; marker of bone resorption); **2)** determine whether preventing the decline in serum iCa during exercise via intravenous Ca administration (i.e., iCa clamp) prevents an increase in serum PTH and CTX; and **3)** measure serum Ca flux and rate of Ca appearance during exercise and determine whether oral Ca loading before exercise attenuates the increase in serum CTX.

BODY

The following **major tasks** were proposed for Year 1:

- **Get regulatory approvals in Q1-2**

The initial local IRB approval was obtained 22 June 2012. The approval was for EXP1 and EXP2-pilot only, because the methods for EXP2 and EXP3 will be finalized after pilot testing is completed. After the initial review by the HRPO, requested modifications to the protocol were approval locally (PAM001-1) on 2 Oct 2012. The protocol was approved by the HRPO on 19 Oct 2012.

Additional minor amendments to the protocol have been approved by the local IRB:

PAM002-1:

This was approved on 14 Jan 2013: **1)** increased total blood volume for EXP1 to 20 mL because the volume listed was an error; and **2)** created a HIPAA Authorization Form A.

PAM003-1:

This was approved on 8 Mar 2013: **1)** clarified that volunteers with abnormal serum 25(OH)D values (<20 ng/mL) at screening will be reconsidered for participation after vitamin D supplementation; **2)** added the possibility of a follow-up visit for a blood draw to recheck abnormal blood results (e.g., vitamin D level) during screening; **3)** increased total blood volume for EXP2-pilot to 60 mL because the volume listed was an error; and **4)** added syncope to the risks of blood draw and IV catheter placement.

PAM004-1:

This was approved 13 Jun 2013 at the time of Continuing Review: **1)** removed Nicole Hirsch as a research assistant and added Toby Wellington; **2)** changed the name of the study physician from Villalon to Shea; **3)** changed the interval between exercise bouts for EXP1 from '4 weeks' to '1 to 4 weeks;' and **4)** modified the protocol for EXP2-pilot to use calcium gluconate when calcium chloride is not available.

The annual Continuing Review of the protocol was approved 7 June 2013.

PAM005-1:

This was approved 17 July 2013: **1)** revised the protocol to return changes to the protocol that were approved in PAM003 (wrong version was submitted for continuing review); **2)** matched language in the protocol to the IRB application form for timing of exercise bouts; **3)** specify that calcium gluconate is not preferred but will be used only when calcium chloride is not available; **4)** added page numbers to the protocol; **5)** corrected the remuneration amounts in the protocol to match the consent forms.

PAM006-1:

This amendment was approved 22 Aug 2013: **1)** added history of asthma as an exclusion criterion; **2)** updated the screening and consent forms to reflect the exclusion for asthma.

- **Hire and train research assistant in weeks Q1-2**

This was accomplished in Q1.

- **Generate forms and tables and create the database in Q1-2**

This was completed in Q2.

- **Conduct recruitment, screening, and testing for EXP1 and EXP2 pilot in Q3-4**

Recruitment for EXP1 and EXP2-pilot commenced in Q1 (November 2012). As of the end of Q4, 18 women and 21 men had provided written informed consent for EXP1; the goal is to have 14 women and 14 men complete EXP1. As of the end of Q4, 10 men provided written informed consent for EXP2-pilot; the goal is to have 4 successful iCa clamps before finalizing the methods for EXP2.

Progress on EXP1 through Q4:

	Enrolled	Screen Failure	Withdrew Before Randomized	Randomized	Withdrew After Randomized	Completed
Women	18	2	1	15	1	13
Men	21	2	3	15	3	12
Total	39	4	4	30	4	25

Reasons for withdrawals:

Screening failures:	TSH out of range (1), steroid use in the past 6 months (2), low vitamin D (1)
Before randomization:	time constraints (2), injury not related to the study (1), stopped responding to correspondence (1)
After randomization:	time constraints (3), stopped responding to correspondence (1)

The progress on EXP1 was delayed because of limited access to the metabolic chamber. Use of the chamber is necessary to control the ambient conditions for the study visits. The scheduling challenges occurred as a result of new projects utilizing this resource, including some that require chamber stays up to 7 consecutive days. To ease the pressure on EXP1, we modified the protocol (PAM4-001) to allow for the two exercise bouts to occur at less than 4-week intervals. The original plan to conduct the exercise visits 4 weeks apart was to facilitate testing women at the same menstrual cycle phase. We do not expect that the failure to accomplish this in a few of the female participants will adversely affect the merit of EXP1.

We expect that the final study visit for EXP1 will occur in November 2013 and that 14 women and 14 men will complete the study. We plan to submit an abstract describing the key results of EXP1 in November 2013 for presentation at the 2014 meeting of the American College of Sports Medicine. Preparation of the first manuscript should start in December 2013.

Progress on EXP2-pilot through Q4 (men only):

Ten men were enrolled, 2 completed the iCa clamp, 1 did not complete the clamp because of an unanticipated problem (bronchospasm), 1 was a screen failure (steroid use in past 6 months), and 3 withdrew (time constraints). Both iCa clamps were successful in preventing a decline in serum iCa during exercise.

We expect that study visits for EXP2-pilot will be completed in October 2013. The clamp methods described in the protocol have been successful. If revisions are necessary, they should be minor. Accordingly, we expect to receive IRB approval for EXP2 in November or December 2012.

Progress on EXP2-pilot was delayed because of a shortage of Ca chloride at University of Colorado Hospital (UCH). The Ca chloride infusate for EXP2-pilot is prepared by the research pharmacy and they will use only product purchased through UCH. Because Ca chloride is used for clinical purposes at UCH, the availability of product for research purposes is restricted during such shortages. The protocol was amended (PAM004-1) to allow the use of Ca gluconate when Ca chloride is not available. Both products have been in short supply, but this is expected to improve in coming months.

We reported one unanticipated problem to the local IRB and the sponsor. The event was a bronchospasm that started approximately 20 minutes after the initiation of the calcium infusion and 5 minutes after the start of exercise. The participant had indicated a negative history of asthma on the medical history form, but acknowledged after the event that this was not correct. The local IRB determined that this event did not meet the definition of an unanticipated problem because it was unexpected but only possibly related to the study intervention and presented minimal risk to the subject. They further determined that calcium may have had some impact on triggering asthma, but that the causal relationship to date has not been conclusive in published studies. Although it was not clear to the committee whether the event was related to the study interventions, the committee requested that the investigators add history of asthma as an exclusion criterion. The rationale for this recommendation was that use of an albuterol inhaler before exercise (which our study physician recommends for participants with a history of asthma) could affect physiologic responses to exercise and introduce a confounding factor to the study. The PI disagreed with this rationale based on published evidence, but complied with the IRB request. The protocol was amended to exclude volunteers with a history of asthma (PAM006-1).

- **Establish and check the quality assurance procedures for the database in Q3-4**

Quality assurance procedures that have been adopted include: a) use of electronic transfer of data to the database whenever possible to avoid manual entry errors; b) use of standardized common data elements (e.g., study ID, sex, age) across multiple data sources to ensure accurate merging within the database; c) independent review of manually entered data; and d) range checks to identify outliers.

- **Prepare annual progress report in Q4**

This was accomplished.

- **Hold medical monitor meeting in Q4**

The medical monitor meeting was scheduled for 14 October 2013.

- **Get regulatory approvals for EXP2 and EXP3-pilot in Q4**

Progress on EXP2-pilot was delayed because of shortages of the product required for the calcium clamp (i.e., calcium chloride or calcium gluconate). Therefore, the application for IRB approval of EXP2 is expected to occur in Year 2 Q1. It is also expected that the application for IRB approval of EXP3-pilot will occur in Year 2 Q1. Advancing this project was temporarily put on hold because of the shortages in the product required (i.e., calcium chloride or calcium gluconate).

KEY RESEARCH ACCOMPLISHMENTS

Because the first experiment has not yet been completed, there are no key accomplishments to report.

REPORTABLE OUTCOMES

Because the first experiment has not yet been completed, there are no reportable outcomes.

CONCLUSION

Because the first experiment has not yet been completed, we cannot yet draw any conclusions.

REFERENCES

The PI is not aware of any published studies that inform this research beyond those included in the grant application.

APPENDICES

none

SUPPORTING DATA

none